56. A transgenic tuber according to claim 53, wherein said gene encodes for a second enzyme.

57. A transgenic seed according to claim 54, wherein said gene encodes for a second enzyme.

58. A process according to claim 2, wherein the fragment is from a plant gene.

59. A process according to claim 2, wherein the fragment is from a non-plant gene.

REMARKS

New claims 31-59 have been entered. The new claims find support throughout the specification; see for example original claims 2, 13 and the Figure 1. A correction in nomenclature has been made, correcting "adenine" to -- adenosine -- as it refers to the enzyme EC 2.7.7.27

Claims 2-4, 7-8, 13-16 and 20-59 are now active in the application.

Applicants acknowledge with appreciation the Examiner's courtesy extended to Applicants and their attorney during a personal interview on June 6, 1996. During the interview, the amendment now proposed was

discussed. The substance of the discussion is set forth below.

Claims 2-4, 7-8, 13-16, 22, 24-26 and 29-30 stand rejected under the judicially created doctrine of obviousness-type double patenting as unpatentable over claim 1 of U.S. Patent No.5,387,756. Reconsideration of the rejection is requested in view of the terminal disclaimer submitted herewith.

Claim 23 stands rejected under 35 USC 112, second paragraph, as being indefinite, being dependent upon cancelled claim 9. It is believed that by the amendment now proposed the objection will be removed. Entry of the amendment is requested with reconsideration of the rejection.

Claims 2-4, 7-8, 13-16, 19 and 21-30 stand rejected under 35 U.S.C. 112, first paragraph. It is alleged that the disclosure is enabling only for claims limited to a process for the introduction of a gene encoding either phosphofructokinase or adenosine diphosphoglucose pyrophosphorylase into the genome of a plant cell. Reconsideration of the rejection is requested for the following reasons.

The claims concern processes for the preparation of transgenic plants by incorporating into a host plant, a chimaeric gene which includes the DNA comprising the

coding sequence encoding for an enzyme selected from the group consisting of:

1. adenosine diphosphoglucose pyrophosphorylase (hereinafter referred to as ("ADPG");
2. acid invertase (hereinafter referred to as "AI");
3. starch synthase;
4. sucrose synthase;
5. pyruvate kinase;
6. 6-phosphofructokinase (pyrophosphate); and
7. sucrose phosphate synthetase.

Applicants' parent application, now issued as U.S. Patent 5,387,756, concerned the DNA fragment which encoded for the enzyme phosphofructokinase for a total of 8 enzymes.

These 8 enzymes, although chemically diverse, are all related in that they function in plant cells to modify the amount of metabolic intermediate in glycolysis or in a pathway for the synthesis or degradation of starch, sucrose or a reducing sugar. The relationship of the 8 enzymes in affecting glycolysis has been set forth in the schematic diagram of Figure 1 in the drawings of applicants' specification. This relationship is well known to the skilled artisan; see for example the Biochemistry of Plants, Vol. 14, Ed. J. Preiss (especially Chapters 1 and 6).

The 8 enzymes are all well known, as are the DNA sequences which encode for their expression in plants. This has been described and set forth in applicants' specification at page 5, second full paragraph and third paragraph bridging page 6.

In the Office Action, it is said that:

> "The disclosure is enabling only for claims limited to transgenic plants comprising either an introduced phosphofructokinase gene or an ADPGPP gene".

However, as mentioned above, Applicants' specification at pages 5 and 6 does indicate that the necessary DNA sequences have already been isolated and are known. The general technique for identifying and isolating particular genes is certainly a known technique; see Khurshead et al., cited and of record in the application.

During the interview with Examiner Fox, he inquired as to whether or not Applicants could show that the coding sequences for each enzyme were available to those skilled in the art at the time the instant application was filed in the U.S. Patent and Trademark Office, (June 7, 1990). The answer is yes. Attached hereto and made a part of this response are seven references relating to the sequences for five of the enzymes and evidencing availability before 1989, as follows.

ADPGPP	1986
Starch Synthase	1988
Sucrose Synthase	1988
Invertase	1985 & 1988
Pyruvate kinase	1985 & 1987

As is disclosed on page 12 of Applicants' specification, the PFK gene was available in 1985.

This thus accounts for six of the eight enzymes and leaves PFP and SPS to be addressed. Enclosed also are referenes evidencing that PFP and SPS were obtained as purified proteins in 1983 and 1989 respectively. From the purified proteins the amino acid sequences could have been ascertained, following which cDNA clones for the respective genes could be obtained or a respective antibody could be used to screen an expression library of cDNA clones. All of this would have been well within the capability of a person of ordinary skill in the art in 1989. The following reference, for example, evidences that methods were available for cDNA cloning of expression libraries.

"Burrell, M.M. Construction of cDNA libraries in λgt10 of λgt11 chapter 19 in Methods in Molecular Biology Vol 4 New Nucleic Acid Techniques edited by J.M. Walker. Humana Press. 1988".

Previously, Applicants have provided evidence of enablement for the invention in respect to PFK and ADPG

(as acknowledged by the Examiner in the Office Action). In addition, before the filing of this application a Journal article had appeared (October, 1990) which indicates that tobacco plants have been transformed with acid invertase (AI) DNA sequences. The transgenic tobacco plants exhibited a new phenotype with accumulated carbohydrate, i.e.; an effect upon glycolysis as applicants herein have proposed. A copy of the article (Antje van Schaewen et al., The Embo Journal Vol. 9, No. 18, pp 3033-3044 [1990]) has been filed in the record. Although the publication is before the filing date of the present application, it is not a prior art reference within the meaning of 35 USC 102 or 103 since it does not antedate Applicants' priority date under 35 USC 120. The publication fully supports and confirms enablement of Applicants' invention in respect of 1) plants other than rice and potato and 2) DNA sequences coding for an enzyme other than PFK and ADPG. In other words, the Journal article further confirms the broad embrace of Applicants' claims 2-4, 7-8, 13-16 and 21-26, by showing that the skill of the art is such that the present invention is enabled with the specification teachings.

Of this argument, made previously in Applicants' parent application, it is said in the Office Action that:

> "The Examiner maintains that von Schaewen et al. demonstrate deleterious effects on plant health following transformation with the acid invertase gene, as well as variability both within and between

plant species, thus demonstrating the unpredictability inherent in the process. Applicants' specification does not provide any guidance regarding the transformation or evaluation of plants with non-exemplified genes."

Applicants cited the von Schaewen et al. paper in support of their contention that it shows that the invention of Claim 2 has application in respect of genes of specified group other than PFK and ADGPP and in respect of plants other than potato. Applicants remain of the belief that the paper fulfills that function. It shows that when tobacco and Arabidopsis plants are transformed with the invertase gene there results for both types of plant an increase in starch; See Tables III and IV of the reference.

As to the phenotypic changes in tobacco which were reported by von Schaewen et al. it is stated on page 3037 that considerable variation was observed between different transformants. Thus, in respect of height variations it is stated that some transformants almost reached the height of a wild-type plant, the latter height being given as about (i.e. variable) 150cm. Again, whereas bleaching and necrotic reactions were observed in respect of older leaves of some plants, for others bleaching was not accompanies by necrotic reactions.

The work of von Schaewen et al was of a strictly scientific nature. Genetic manipulation techniques were employed for the purpose of enhancing knowledge of sink-

source relationships. However, to the skilled in the art addressee interested in a practical sense in effecting metabolic changes in plants, the major teaching of the von Schaewen et al. paper is that a process as per Claim 2 works effectively in respect of a sequence coding for acid invertase. In regard to tobacco, such person would readily observe the variation in the phenotypic effects and that by virtue of such variation there was open to him the opportunity of selecting, according to his specific requirements, individuals which are little effected. From such individuals further individuals could be produced by cloning or crossing. It should also be noted that the said specific requirements may be many and various, and that according to some at least of these a phenotypic change, or a change present to a lesser degree, may be of little or no consequence, either in absolute terms or because the positive payoff of using the inventive method predominates.

Proposals have long been made for growing tobacco for the purpose of providing a source of food protein. As can be observed from Table 6B of the von Schaewen et al paper, there is a prospect of harvesting tobacco leaves having an enhanced level of protein.

In any event, applicants have by their own work confirmed the promise of von Schaewen et al. Attached hereto and made a part of the response is a Declaration of

the co-inventor, Burrell, setting forth details of experiments to demonstrate enablement of the present invention.

In a first experiment, potato plants were transformed with a chimaeric gene comprising a patatin promoter, an antisense invertase coding sequence and a nos terminator. The bar chart set forth in the Declaration shows results, in terms on the Y-axis, of the ratio sucrose glucose + fructose. (On the chart "sem"=standard error of the mean).

The bar chart clearly shows that plants were obtained with a greatly increased ratio of sucrose to glucose + fructose. This is a commercially advantageous attribute in certain forms of potato processing. The person skilled in the art could readily select such plants and clonely multiply them, so to obtain commercial quantities thereof.

In a second procedure, potato was transformed with the chimaeric gene as above described and nine independent transformed lines were grown in a paired experiment with each line replicated ten times. As is shown by the results table in the Declaration, the reducing sugar content, as represented by the content of glucose, is significantly less in the transgenic plants than in control plants, whereas in the transgenic plants the sucrose-glucose ratio is significantly increased relative to the control plants.

In a second experiment, a field trial was conducted with potatoes transformed with the sequence for sucrose synthase in a chimaeric gene which also comprised a patatin promoter and a nos terminator. The trial was of a randomized block design and there were three replicates. An analysis of variance in regard to the specific gravity (an indicator of starch content) for the three lines indicates that they were significantly different from controls (standard error of difference = 0.0075).

Line	Specific Gravity
89	1.168
36	1.145
52	1.137
Control	1.117

In a third experiment, the coding sequence for the wheat homolog of waxy was used to transform a hybrid maize. The seed from progeny plants obtained in the transformation demonstrated expression of the anti-sense wheat waxy gene by reduced amylose production.

Finally, in a fourth experiment, the anti-sense sequence for sucrose phosphate synthase from spinach was substituted for PFK in a vector. The chimaeric gene was then introduced into potato and plants regenerated. The experimental results clearly show the impact on sugar production.

Thus, the applicability of the invention has been shown for five plants (potato, rice, tobacco, maize and Arabidopsis thaliana) and in respect of six enzymes.

For the above reasons, it is seen that the von Schaewen et al. paper evidences wide applicability of the invention and that it rebuts any position of unpredictability. Phenotypic reactions are a separate issue from workability (starch did increase). Furthermore, as outlined above, such reactions may, in particular circumstances, be acceptable, may be rectified or may be avoided.

It is clear that undue experimentation is not required, to identify and isolate the gene or genes which encode any other enzyme of carbohydrate metabolism and to evaluate the effects of said gene(s) on transformed plant cells and plants.

Clearly, Applicants have met the mandate of 35 USC 112, first paragraph, which is to teach how to "make and use" the invention. The enablement requirement need not be met with specific working examples (In re Borkowski, 164 USPQ 642) but by consideration of the specification as a whole. The question of predictability has been answered in Applicants' favor by the appearance of the von Schaewen publication. The claims are process claims and the products of those processes. Practice of these processes do not require undue experimentation. There is "insufficient unpredictability" within the meaning of the Board of Patent Appeals and Interferences' decision in the case of Ex Parte King, 17 USPQ 2nd 1545. Those skilled in

the art with the evidence shown by the actual use of the genes for expressed enzymes and knowledge of the metabolic pathways shown in the drawing of Applicants' Fig. 1, would expect the operative effect of transgenesis with the remaining 2 enzymes.

Claim 30 stands rejected under 35 USC 102(b) as anticipated by Grill et al. Reconsideration of the rejection is requested for the following reasons.

Claim 30 is directed to a transgenic plant, which contains a chimaeric gene including a DNA fragment which comprises a coding sequence for an enzyme in the pre-existing plant pathway of glycolysis or the synthesis or degradation of starch, sucrose or a reducing sugar. Grill et al. describes a transgenic plant containing a chimaeric gene including a DNA fragment which comprises a coding sequence for cyclodextrin glucotransferase which establishes a new metabolic pathway to degrade starch. Thus, Grill et al. does not contemplate modification of an existing metabolic pathway, thereby affecting glycolysis or synthesis or degradation of starch, sucrose or a reducing sugar.

Claim 30 was also rejected under 35 USC 103 as unpatentable over Houck et al. taken with Gay et al. Reconsideration of the rejection is requested for the following reasons.

Claim 30 has been described above, and clearly pertains to modification of a metabolic pathway pre-existing in the plant.

Gay et al. teach the addition of DNA sequence coding for an enzyme of bacterial origin, which is not found in plants. Clearly, the combination suggested of Gay et al's teachings with Houck et al. would not show or suggest modification of a pre-existing (in the plant metabolic pathway. Reconsideration of the rejection is requested in view of the amendment to claim 30 now made.

Claims 2-4, 7-8, 13-16, 21-24 and 30 stand rejected under 35 U.S.C. 103 as being unpatentable over Twell et al. taken with de Graaff et al., Ap-Rees et al. and Yang et al. Reconsideration of the rejection is requested for the following reasons.

Claim 30, the method claim of broadest scope, is directed to a method for the preparation of a transgenic plant. In accordance with the method, a plant cell is transformed with a chimaeric gene comprising a promoter and a gene encoding for a polypeptide having the activity of an enzyme which regulates the amount of a metabolic intermediate in glycolysis or in a pathway for the synthesis or degradation of starch, sucrose or reducing sugar from a glycoyltic intermediate.

It is submitted that none of the cited prior art references, alone or in any combination, show or suggest

the invention of Claim 2 and the unexpected findings attaching thereto. More particularly, Twell et al. teaches that the potato may be transformed with the tuber specific patatin promoter and a chimaeric gene for patatin. As recognized by the Examiner, Twell et al. is not concerned with and does not suggest any other gene or its expression in a transgenic plant.

deGraaf et al. is cited only for the disclosure of the cloning of a fungal pyruvate kinase gene and its expression in a host.

Ap-Rees et al. teaches the relationship between sucrose accumulation in potato tuber and loss of PFK activity and the undesirability of sucrose accumulation in cold-stored potato.

Yang et al. is cited for their teaching of

"transformation to alter potato tuber quality"

and their suggestion of the patatin promoter (the patatin promoter was suggested, but not used, as possibly improving the yields of protein which might be expressed by the DNA sequence coding for a protein high in essential amino acids). Nothing was suggested concerning carbohydrate metabolism.

Based on the four cited references, it was concluded in the Office Action that:

> It would have been obvious to one of ordinary skill in the art to utilize the method of potato transformation taught by Twell et al., and to modify that method by incorporating the

> pyruvate kinase structural gene taught by deGraaff et al.; given the teaching by Ap-Rees et al. of the relationship between tuber sweetening and pyruvate kinase activity, the suggestion by Yang et al. to utilize plant transformation for potato tuber quality improvement, and the recognition by those of ordinary skill in the art that each would have continued to function in its known and expected manner. Thus, the claimed invention was clearly prima facie obvious as a whole to one of ordinary skill in the art at the time it was made, especially in the absence of evidence to the contrary."

In other words, Twell et al. and deGraaff et al. are representative of the technique for preparing transgenic plants. Applicants agree that the techniques of preparation are indeed "state of the art". However, obviousness under 35 USC 103 can only be determined when the prior art is considered as a whole, including the differences between the prior art and the claimed invention. In the decision of the Federal Circuit Court of Appeals rendered in Northern Telecom, Inc. V. Datapoint Corp., 15 USPQ 2d 1321 the court found that the nature of the problem which persisted in the prior art and the inventor's solution to the problem must be considered in determining prima facie obviousness.

Thus, although the techniques of preparing a transgenic plant are known, where does the cited prior art suggest that the process of the invention will solve, for example, the problem of sugar accumulation in the tuber of a potato plant?

Ap-Rees does not concern transgenic plants. Ap-Rees concerns cold-lability of the PFK forms. Ap-Rees offers no motivation to reduce sugar accumulations by enhancement of PFK activity by any means whatsoever, especially by genetic manipulation.

Yang et al. does not fill the void of Ap-Rees. The Yang et al paper is merely representative of the common general knowledge. All it provides is an exemplification of the fact that techniques are available whereby genes can be transferred into plants. The commonality of plant type (potato) between Yang et al and Ap Rees et al is, in the present context, of no import. The Examiner mentions the fact that Yang et al were seeking to modify plant quality. This is a feature of all commercially orientated work involving the genetic modification of plants. In other words, the citation of Yang et al merely exemplifies plant improvement by genetic manipulation.

Furthermore, as stated by Judge Rich in the decision of In re Vaeck, 20 USPQ 2d 1438:

> "Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under Section 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should *** carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable

> expectation of success. [citations] Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicants' disclosure." (underlining added)

Both of the required elements are lacking in the prior art cited in the application at bar, particularly in respect to an expectation of a solution of the prior art problem.

The invention is particularly applicable to potatoes. It had been expected that the introduction and expression of additional pyruvate kinase into potato tuber cells would cause a high flux in the glycolytic pathway. Flux is defined as is defined as the rate at which chemical compounds are converted from one compound to another in a pathway. A metabolic intermediate is a chemical compound in the pathway which is converted into something else (metabolism being the process of converting one chemical into another).

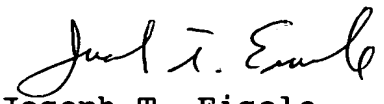
For all of the above reasons, it is submitted that claims 2-4, 7, 13-16 and 21-24 are all patentably distinguished from the cited prior art and allowable.

The newly entered claims 31-59 are all narrower in scope and directed to embodiments of claims 2 or 13. It is submitted that claims 31-59 are all patentably distinguished for the same reasons given above to distinguish claims 2 and 13.

Applicants acknowledge with appreciation the Examiner's indication that claims 20, 25-26 and 27-29 are free of the prior art and the allowance of claim 20.

A speedy and favorable reconsideration of the rejection is requested, together with consideration of new claims 31-59, inclusive.

Respectfully submitted,



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